STIC-ILL

ADONIS; QR180.I54

From: Huynh, Phuong N.
Sent: Thursday, February 13, 2003 4:37 PM
To: STIC-ILL
Subject: RE: 09/852,976

Importance: High

Please deliver the following:

International Immunology 9(11): 1767-72, 1997

J Immunol 170(3): 1158-66; 2003

Thanks,
Neon
Art unit 1644
Mail 9E12
Tel 308-4844

International Immunology, Vol. 9, No. 11, pp. 1767–1772

CD79α/CD79β heterodimers are expressed on pro-B cell surfaces without associated μ heavy chain

Mariko Koyama, Katsuhiko Ishihara[1], Hajime Karasuyama[2], Jacqueline L. Cordell[3], Aikichi Iwamoto and **Tetsuya Nakamura**

Department of Infectious Diseases and Applied Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan
[1]Department of Molecular Oncology, Biomedical Research Center, Osaka University Medical School, Osaka 565, Japan
[2]Department of Immunology, The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan
[3]LRF Immunodiagnostics Unit, University Department of Cellular Science, John Radcliffe Hospital, Oxford, UK

Keywords: B lymphocytes, B cell differentiation

Abstract

During B cell development, the surface expression of CD79α/CD79β heterodimers had been thought to begin in the pre-B cell stage where the heterodimers constitute pre-B cell receptors together with μ heavy and surrogate light chains. Thereafter, in mature B cells, CD79α/CD79β associates with surface Ig to form B cell antigen receptors. In this study, we revealed by using newly established mAb that CD79β was expressed on the surface of pro-B cells which had not undergone the productive Ig gene rearrangement. Biochemical analysis showed that CD79β on pro-B cells existed either as monomers or as disulfide-linked heterodimers with CD79α, non-covalently associated with four unidentified membrane molecules. Our finding that CD79β is expressed on earlier B-lineage cells than previously expected coincides with the recent study in which CD79β-deficient mice exhibit a blockade of B cell differentiation at the pro-B cell stage. Thus, it is speculated that the CD79β-containing complexes on pro-B cell surfaces may function to induce early B cell differentiation.

Introduction

B cells express antigen receptors (B cell receptor; BCR) which consist of surface μ heavy chain (HC), light chain (LC) and CD79α/CD79β heterodimers (1). The physical association of CD79α/CD79β heterodimers with μHC is necessary for the efficient transport of μHC to cell surfaces as well as for signal transduction through the BCR (2). In spite of such close relationship between CD79α/CD79β heterodimers and μHC, they exhibit a distinct pattern of cytoplasmic expression during B cell differentiation. The cytoplasmic expression of CD79α/CD79β begins at the pro-B cell stage in which the productive Ig gene rearrangement is not yet accomplished, whereas that of μHC is delayed until the pre-B cell stage (3,4). Since previous studies using mAb to human CD79β (3,5) and polyclonal antibody to murine CD79β (6) could not convincingly detect CD79β on pro-B cell surfaces, it had been speculated that CD79β was expressed in the cytoplasm of pro-B cells but stayed there until μHC was synthesized in the pre-B cell stage to form the pre-B cell receptor together with surrogate LC. Consequently, pre-B cells had been considered the earliest B-lineage cells to express cell surface CD79α/CD79β.

In this paper, however, we show the expression of a small but significant amount of CD79β on pro-B cell surfaces by using newly established mAb to murine CD79β. The biochemical analysis revealed that CD79β either existed as monomers or constituted heterodimers with CD79α on pro-B cell surfaces and was non-covalently associated with four membrane molecules distinct from μHC. Possible functions of CD79β on pro-B cell surfaces will be discussed.

Correspondence to: T. Nakamura

Transmitting editor: T. Watanabe

Received 10 July 1997, *accepted* 11 August 1997

Table 1. Cell surface expression of CD79β on B-lineage cells

Cell line	HC gene[b]	Surface expression (sMFI [a]) of			
		CD79β	λ_5	$V_{pre\text{-}B}$	μHC
63-24	G	12	18	3	0
40E1	DJ/DJ	23	10	3	0
38B9	DJ/DJ	31	32	7	0
230-238	VDJ^-/VDJ^-	3	1	0	0
220-8	VDJ^-/VDJ^-	9	7	2	0
NFS5.3	R	17	73	15	11
1A9	R	24	363	143	88
WEHI231	R	654	0	0	2612
Bal17	R	270	0	0	362

[a]Specific mean fluorescence intensity: MFI of staining with specific mAb – MFI of staining with control antibody.

[b]Gene rearrangements of Ig HC (VDJ^-, non-productive $V_HD_HJ_H$ rearrangement; DJ, D_HJ_H rearrangement; R, productively rearranged; G, germline).

Methods

Cells and antibodies

Murine B-lineage cell lines used in this study are listed in Table 1 and were described previously (6–8). Purified or peroxidase-labeled goat antibody to hamster IgG or mouse Ig, and peroxidase- or phycoerythrin (PE)-labeled streptavidin were purchased from Southern Biotechnology (Birmingham, AL). Red670-labeled streptavidin was from Gibco/BRL (Grand Island, NY). FITC-labeled, PE-labeled, biotinylated or purified mAb to CD19 (1D3), BP-1 (6C3), μHC (AM/3), CD43 (S7), CD45R (RA3-6B2), $V_{pre\text{-}B}$ (VP245), λ_5 (LM34) and the synthetic peptide of CD79α (HM57) were described previously (4,8,9).

Establishment of mAb to CD79β

CD79α/CD79β heterodimers were purified from WEHI231 B cells as described previously (3) and used for immunization of footpads of an Armenian hamster. After the last immunization, regional draining lymph nodes were excised and lymphocytes were fused with X63-Ag8.653 (3). Hybridoma supernatants were initially screened for positive reactivity in surface immunofluorescence of WEHI231 and then the specificity to CD79α/CD79β heterodimers was tested by immunoprecipitation using digitonin lysates of surface-labeled WEHI231. As a result, from ~200 hybridomas, mAb secreted by five hybridomas turned out reactive with the extracellular portions of CD79α/CD79β heterodimers. After several rounds of limiting dilutions, clones which stably produced mAb were obtained from the five candidates and two of them (designated HM79-12 and HM79-16) were further analyzed for the specificity to CD79β (see Results).

Biochemical analysis

For immunoprecipitation of membrane proteins, cells were surface biotinylated and lysed in 1% NP-40 or digitonin lysis buffer (3). Cell lysates were incubated with Sepharose 4B coupled with goat antibody to mouse Ig or Protein G–Sepharose (Pharmacia Biotech, Tokyo, Japan) preincubated with HM79-12, HM79-16 or normal hamster IgG. After washing, the immunoprecipitates were eluted, separated by one-dimensional SDS–PAGE or non-reducing/reducing two-dimensional SDS–PAGE and transferred onto Immobilon-P membranes (Millipore, Bedford, MA). The biotinylated proteins were detected by incubating the membranes with peroxidase-labeled streptavidin and visualized by the ECL system (Amersham, Arlington Heights, IL). For Western blot analysis, immunoprecipitates were prepared from unlabeled cells, separated by two-dimensional SDS–PAGE and transferred onto membranes. The membranes were incubated with HM57 or HM79-16 and the bound antibodies were detected by peroxidase-labeled goat anti-mouse Ig or peroxidase-labeled goat anti-hamster Ig respectively, followed by the ECL system.

Immunofluorescence analysis

For the staining of cell lines, cells (1×10^6) were incubated with biotinylated antibodies (50 μg/ml) for 20 min and the bound antibodies were detected with PE-labeled streptavidin (20 μg/ml). Biotinylated normal hamster or rat IgG served as controls. For the staining of bone marrow cells derived from 4- to 6-week-old BALB/c mice, surface μHC^+ cells were eliminated by panning with Petri dishes coated with anti-IgM antibody and the $CD45R^+$ cells were then enriched with MiniMACS (Miltenyi Biotec, Gladbach, Germany) as described previously (9). The purified cells were stained with 50 μg/ml of FITC-labeled, PE-labeled and biotinylated mAb, and the biotinylated mAb was developed with Red670-labeled streptavidin. Biotinylated normal hamster IgG was used as a control of biotinylated HM79-12. The indirect detection of anti-CD45R mAb was described previously (9). Cells were analyzed on a FACScan or FACSort using Lysys II software (Becton Dickinson, San Jose, CA).

Results

HM79 recognizes extracellular epitopes of murine CD79β

As described in Methods, two mAb, HM79-12 and HM79-16, were selected based upon the reactivity with the extracellular portions of CD79α/CD79β heterodimers. From digitonin lysates of surface-biotinylated WEHI231 B cells, anti-Ig antibody co-precipitated CD79α (arrowhead) and CD79β (arrows) together with μHC and κLC in reducing conditions (Fig. 1A, panel 1, lane 4) as described previously (6). When HM79-12 and HM79-16 were used for immunoprecipitation from the same lysates, the immunoprecipitated molecules appeared to be compatible to those by anti-Ig antibody (Fig. 1A, panel 1). However, the result was quite different when cells were lysed in harsh detergent NP-40 which is known to dissociate the non-covalent association between CD79α/CD79β heterodimers and μHC. From NP-40 lysates, both HM79 mAb immunoprecipitated CD79α and CD79β without μHC and κLC in reducing conditions and 80–90 kDa of CD79α/CD79β heterodimers in non-reducing conditions (Fig. 1A, panels 2 and 3). In contrast, anti-Ig antibody immunoprecipitated μHC and κLC alone in reducing conditions and the IgM in non-reducing conditions from the same NP-40 lysates (Fig. 1A, panels 2 and 3), indicating that the specificity of HM79 was restricted to either CD79α or CD79β. To further determine the specificity of HM79, immunoprecipitation and Western blot analysis were performed. From

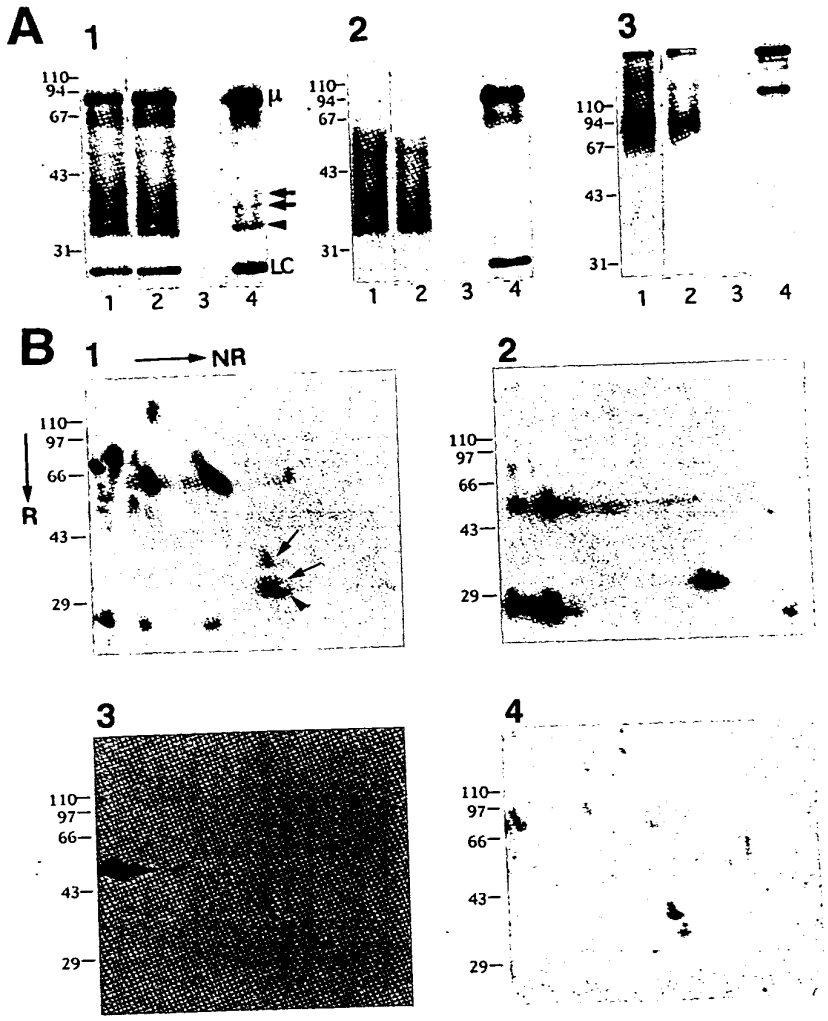

Fig. 1. HM79-12 and HM79-16 are specific for murine CD79β. (A) WEHI231 B cells were surface biotinylated, lysed in digitonin (panel 1) or NP-40 (panels 2 and 3) lysis buffer and immunoprecipitated with HM79-12 (lane 1), HM79-16 (lane 2), normal hamster IgG (lane 3) or goat anti-mouse Ig antibody (lane 4). Immunoprecipitates were resolved by 10% SDS–PAGE under reducing (panels 1 and 2) and non-reducing (panel 3) conditions, transferred to membrane, and biotinylated proteins were visualized. Indicated in lane 4 in panel 1 are μHC (μ), CD79β (arrows), CD79α (arrowhead) and LC. (B) Surface biotinylated (panel 1) or unlabeled (panels 2–4) spleen cells were lysed in digitonin lysis buffer and immunoprecipitated using goat antibody to mouse Ig. The immunoprecipitates were resolved by non-reducing (10%)/reducing (10%) two-dimensional SDS–PAGE and transferred to membranes. In panel 1, the biotinylated proteins were visualized with the ECL system. In panels 2–4, membranes were incubated with anti-CD79α mAb HM57 (panel 2), HM79-12 (panel 3) or HM79-16 (panel 4), and the bound antibodies were detected by appropriate second antibodies.

digitonin lysates of surface-biotinylated spleen B cells, anti-Ig antibody precipitated two spots of differentially glycosylated CD79β (Fig. 1B, panel 1, arrows) and one spot of CD79α (arrowhead) together with Ig HC and LC (6). When the immunoprecipitates from unlabeled spleen B cells were subjected to Western blot analysis, the mAb to CD79α (HM57) recognized the smaller CD79α spot, whereas both HM79-12 and HM79-16 reacted with the larger two spots (Fig. 1B, panel 2–4), indicating that the HM79 mAb were specific to CD79β. Off-diagonal spots between the 97 and 43 kDa size markers are murine Ig HC which are cross-reactive with biotinylated goat anti-hamster Ig antibody. Since HM79 are reactive with viable B cells in immunofluorescence, we conclude that HM79-12 and HM79-16 recognize the extracellular epitopes of murine CD79β.

Pro-B cell lines and normal pro-B cells in bone marrow express surface CD79β

We next conducted the immunofluorescence analysis using HM79 with various developmental stages of B-lineage cells, and found that mature B cell Bal17, immature B cell WEHI231, and pre-B cells NFS5.3 and 1A9 (μHC^+, $V_{pre\text{-}B}{}^+$ and $\lambda_5{}^+$)

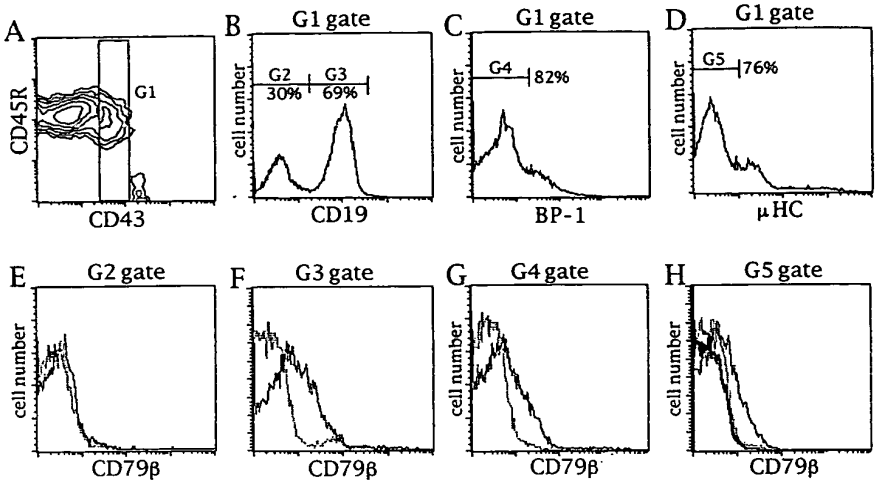

Fig. 2. CD79β expression on pro-B cell surfaces in normal bone marrow. μHC$^-$CD45R$^+$ B progenitor cells were purified from bone marrow. (A) Cells were stained with mAb to CD45R and PE-labeled mAb to CD43, and the CD43$^+$ G_1 gate was determined as a pro-B cell-enriched population. (B–H) Cells were stained with PE-labeled mAb to CD43, FITC-labeled mAb to either CD19 (B, E and F), BP-1 (C and G) or μHC (D and H), and biotinylated mAb to CD79β (HM79-12). The expression of CD19, BP-1 or μHC in the G_1 gate was examined, and the G_2–G_5 gates were determined as described in histograms (B–D). Then, CD79β expression in CD19$^-$CD43$^+$ cells (E, G_2 gate), CD19$^+$CD43$^+$ cells (F, G_3 gate), BP-1$^-$CD43$^+$ cells (G, G_4 gate) and μHC$^-$CD43$^+$ cells (H, G_5 gate) was shown (solid lines). Dotted lines represent the controls stained with biotinylated normal hamster IgG. In (H), binding of biotinylated HM79-12 to μHC$^-$CD43$^+$ fraction (G_5 gate) in the presence of 10 times excess concentration of unlabeled HM79-12 is represented (bold line).

were all positive for surface immunofluorescence of HM79 (Table 1). In contrast to previous studies (3,5,6), however, out of five murine pro-B cells (230-238, 220-8, 63-24, 40E1 and 38B9), none of which had accomplished productive Ig HC gene rearrangements or expressed cytoplasmic μHC (7,8), all showed a small, but significant, amount of cell surface expression of CD79β (Table 1). All non-B lineage cell lines which we examined (P388D1, P815, BW5147 and Jurkat) were completely negative for HM79 staining (data not shown).

Since the results obtained from cell lines were unexpected, we also examined bone marrow pro-B cells for surface expression of CD79β. Surface μHC$^-$CD45R$^+$ B progenitor cells were enriched from bone marrow, and stained for CD43 and CD45R. Figure 2(A) showed that the CD43$^-$CD45R$^+$ B progenitor population could be gated by simply setting the gate on CD43$^+$ cells (G_1 gate). In this G_1 gate, 69% of cells were CD19$^-$ (Fig. 2B), 82% were BP-1$^-$ (Fig. 2C) and 76% were μHC$^-$ (Fig. 2B), indicating that the major population of the G_1 gate had the pro-B cell phenotype (10,11). As shown in Fig. 2(E and F), the significant shift of the histograms by HM79-12 staining was observed in CD19$^+$ cells (G_3 gate) but not in CD19$^-$ cells (G_2 gate), which indicated that the surface expression of CD79β was restricted to B-lineage cells in the G_1 gate. Since CD19$^+$ cells in the G_1 gate had been shown to include pro-B cells and some early pre-B cells (10,11), we then examined the expression of CD79β in the BP-1$^-$ fraction of the G_1 gate (Fig. 2C, G_4 gate) which did not include pre-B cells (10). Figure 2(G) clearly showed that the BP-1$^-$ pro-B cell fraction expressed CD79β. Although the G_1 gate was contaminated with μHC$^+$ cells (Fig. 2D), we could confirm that μHC$^-$ cells in the G_1 gate (G_5 gate) expressed surface CD79β (Fig. 2H, solid line). In addition, 10 times excess amount of unlabeled anti-CD79β mAb completely inhibited the binding of the biotinylated mAb to μHC$^-$CD43$^+$ (Fig. 2H, bold line), proving the specific binding of the biotinylated mAb. From these results we conclude that bone marrow pro-B cells express surface CD79β.

Biochemical analysis of CD79β on pro-B cell surfaces

On mature B cell surfaces, CD79β constitutes heterodimers with CD79α via disulfide linkage and the heterodimers are non-covalently associated with IgM to form a stable BCR (1). Thus, it is of great interest to know how CD79β is expressed on cell surfaces of pro-B cells without IgM. To elucidate this point, we conducted biochemical analysis using 38B9 pro-B cells which showed the highest expression of surface CD79β in pro-B cell lines (Table 1). After cells were surface biotinylated and lysed in digitonin, CD79β and the associated molecules were immunoprecipitated by anti-CD79β mAb. As shown in Fig. 3(A), reducing/non-reducing two-dimensional SDS–PAGE showed that the immunoprecipitates were resolved into two off-diagonal spots and six on-diagonal spots. The off-diagonal spots appeared to be generated from the heterodimeric complex with disulfide linkage, and the Western blot analysis using anti-CD79α mAb (HM57) and anti-CD79β mAb (HM79-16) (Fig. 3B and C) revealed that the larger spot (Fig. 3A, arrow) was CD79β and that the smaller one (Fig. 3A, arrowhead) was CD79α. Two spots on the diagonal line (Fig. 3A, arrow and arrowhead) were also CD79β and CD79α as demonstrated in Fig. 3(C) and the longer exposure of Fig. 3(B) (not shown). Anti-CD79β mAb precipitated four spots on the diagonal line which reacted with neither anti-CD79α mAb nor anti-CD79β mAb (Fig. 3A). The estimated molecular sizes of the four spots were 110, 90–60, 30 and

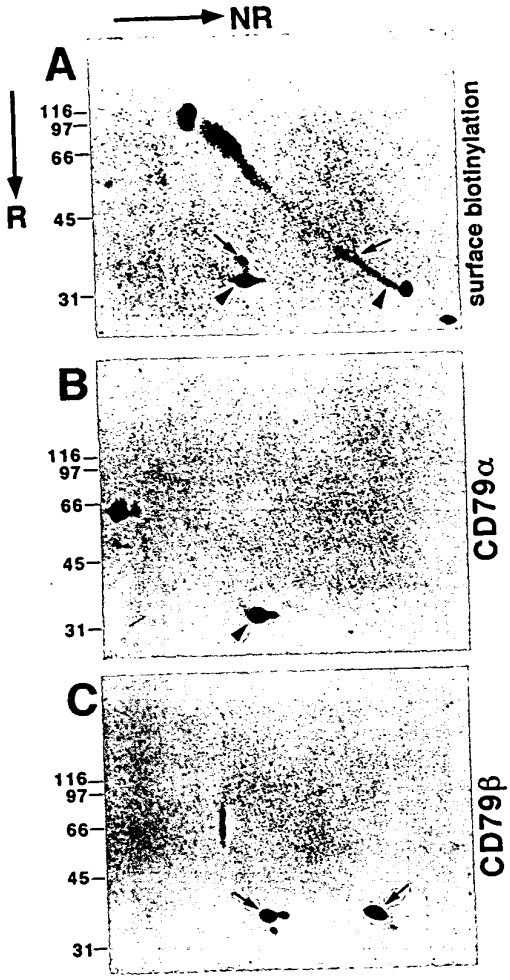

Fig. 3. Biochemical analysis of CD79β on pro-B cells. (A) 38B9 pro-B cells were surface-biotinylated, lysed in digitonin and immunoprecipitated with anti-CD79β mAb (HM79-16). The immunoprecipitates were resolved by non-reducing (10%)/reducing (10%) two-dimensional SDS–PAGE, transferred to Immobilon P membranes and the biotinylated molecules were visualized. Arrows and arrowheads indicate the spots reactive with anti-CD79β and CD79α mAb in (B) and (C) respectively. (B and C) Immunoprecipitates using anti-CD79β mAb from digitonin lysates of unlabeled 38B9 pro-B cells were resolved by two-dimensional SDS–PAGE and transferred to membranes. The membranes were incubated with anti-CD79α mAb HM57 (B) or anti-CD79β mAb HM79-16 (C) and bound mAb were detected as described in the legend to Fig. 1. Arrowhead and arrows indicate the spots which were specifically reactive with each mAb.

<30 kDa, which migrated together with the front line of the gel. All migrated on the diagonal line, indicating the non-covalent association with CD79β.

Discussion

By using novel mAb HM79 which recognize murine CD79β, we here show the first evidence that pro-B cells expressed CD79β on cell surfaces without associated μHC. The specificity of HM79 was biochemically confirmed using immunoprecipitation and Western blot analyses. The comparison of the results of HM79 with those of the mAb to murine CD79α enabled us to conclude that HM79 was specific to murine CD79β. We also established transfectants expressing the murine CD79β gene and ensured the specific binding of HM79 to the transfectants (data not shown).

We found the unexpected surface expression of CD79β not only on pro-B cell lines but also on normal pro-B cells in murine bone marrow. In humans, in spite of the intensive studies using monoclonal reagents, we and others could not detect the surface CD79β on pro-B cells (3,5). Although we do not fully understand the reason for the discrepant results between mice and humans, one simple explanation could be that the regulation of CD79β expression during B cell differentiation differs between the species. In fact, Lassoud *et al.* have biochemically shown that CD79α and CD79β are rapidly degraded in the endoplasmic reticulum of human pro-B cells and fail to reach the cell surface (12). Another possibility is that the antigenic epitopes on CD79β recognized by mAb other than HM79 are accessible when CD79β is associated with μHC, but not accessible when expressed on pro-B cell surfaces together with molecules distinct from μHC. A similar phenomenon has been reported in the case of mAb to surrogate LC (13).

Biochemical analysis revealed that CD79β on 38B9 pro-B cells existed either as monomers or as heterodimers together with CD79α and was non-covalently associated with four membrane molecules to constitute a cell surface complex on pro-B cells. We also showed that not only CD79β but also a small amount of CD79α was expressed as monomers on cell surfaces. The existence of monomeric CD79α has been also reported in murine pre-B cells (14), which may suggest that immature B cells are impaired in the ability to make disulfide linkages. We showed that the anti-CD79β mAb immunoprecipitated monomeric or heterodimeric CD79α/CD79β as well as four other membrane molecules from pro-B cell lysates. However, it is not clear at this moment whether all of them constitute a single cell surface complex or they constitute heterogeneous complexes. Since 38B9 pro-B cells do not express Ig HC and LC, the four molecules associated with CD79α/CD79β are neither HC nor LC. The candidates for the identities of these four membrane molecules would include calnexin, CD22, surrogate LC or other unidentified molecules and further studies are in progress regarding this point.

Gong *et al.* recently showed that CD79β-deficient mice exhibited a complete block in pro-B cell development before the rearrangement of V_H to DJ_H (15). Although their finding was unexpected since CD79β had been thought to be expressed first on cell surfaces of pre-B cells, our results shown here might give some clue to understand their finding. We revealed that CD79β constituted novel complexes on pro-B cell surfaces. As the cross-linkage of the pre-BCR induces LC gene rearrangement in pre-B cells (16), it is possible that signals delivered through CD79β on pro-B cells induce the recombination of V_H to DJ_H, resulting in differentiation of pro-B cells into pre-B cells. If this is the case, the disruption of the CD79β gene would result in a block of differentiation from pro-B to pre-B cells as reported by Gong *et al.* Thus, the identities and functions of the molecules which are non-

covalently associated with CD79β on pro-B cells are of great interest to understand early B cell development, and are now under our investigation.

Acknowledgement

This work was supported by grants from the Ministry of Education, Culture and Science (06770838 and 08839001). We thank Ms Fumiko Ohtani for excellent technical assistance.

Abbreviations

BCR	B cell receptor
HC	heavy chain
LC	light chain
PE	phycoerythrin

References

1 Reth, M. 1992. Antigen receptors on B lymphocytes. *Annu. Rev. Immunol.* 10:97.
2 Venkitaraman, A. R., Williams, G. T., Dariavach, P. and Neuberger, M. S. 1991. The B-cell antigen receptor of the five immunoglobulin classes. *Nature* 352:777.
3 Nakamura, T., Kubagawa, H. and Cooper, M. D. 1991. Heterogeneity of immunoglobulin-associated molecules on human B cells identified by monoclonal antibodies. *Proc. Natl Acad. Sci. USA* 89:8522.
4 Mason, D. Y., Cordell, J. L., Tse, A. G. D., M. van Dongen, J. J., van Noesel, C. J. M., Micklem, K., Pulford, K. A. F., Valensi, F., Comans-Bitter, W. M., Borst, J. and Gatter, K. C. 1991. The IgM-associated protein mb-1 as a marker of normal and neoplastic B cells. *J. Immunol.* 147:2474.
5 Okazaki, M., Luo, Y., Han, T., Yoshida, M. and Seon, B. K. 1993. Three new monoclonal antibodies that define a unique antigen associated with prolymphocytic leukemia/non-Hodgkin's lymphoma and are effectively internalized after binding to the cell surface antigen. *Blood* 81:84.
6 Ishihara, K., Wood, W. J., Jr, Damore, M., Hermanson, G. G., Wall, R. and Kinkade, P. W. 1992. B29 gene products complex with immunoglobulins on B lymphocytes. *Proc. Natl Acad. Sci. USA* 89:633.
7 Frederick, W. A., Yancopoulos, G. D., Blackwell, T. K., Wood, C., Thomas, E., Boss, M., Coffman, R., Rosenberg, N., Tonegawa, S. and Baltimore, D. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. *EMBO J.* 3:1209.
8 Karasuyama, H., Rolink, A. and Melchers, F. 1993. A complex of glycoproteins is associated with VpreB/lambda 5 surrogate light chain on the surface of μ heavy chain-negative early precursor B cell lines. *J. Exp. Med.* 178:469.
9 Ishihara, K., Kobune, Y., Okuyama, Y., Itoh, M., Lee, B. O. K., Muraoka, O. and Hirano, T. 1996. Stage-specific expression of mouse BST-1/BP-3 on the early B and T cell progenitors prior to gene rearrangement of antigen receptor. *Int. Immunol.* 8:1395.
10 Hardy, R. R., Carmack, C. E., Shinton, S. A., Kemp, J. D. and Hayakawa, K. 1991. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J. Exp. Med.* 173:1213.
11 Krop, I., de Fougerolles, A. R., Hardy, R. R., Allison, M., Schlissel, M. S. and Fearon, D. T. 1996. Self-renewal of B-1 lymphocytes is dependent on CD19. *Eur. J. Immunol.* 26:238.
12 Lassoued, K., Illges, H., Benlagha, K. and Cooper, M. D. 1996. Fate of surrogate light chains in B lineage cells. *J. Exp. Med.* 183:421.
13 Sanz, E. and de la Hera, A. 1996. A novel anti-Vpre-B antibody identifies immunoglobulin-surrogate receptors on the surface of human pro-B cells. *J. Exp. Med.* 183:2693.
14 Matsuo, T., Kimoto, M. and Sakaguchi, N. 1991. Direct identification of the putative surface IgM receptor-associated molecule encoded by murine B cell-specific mb-1 gene. *J. Immunol.* 146:1584.
15 Gong, S. and Nussenzweig, M. C. 1996. Regulation of an early developmental checkpoint in the B cell pathway by Igβ. *Science* 272:411.
16 Tsubata, T., Tsubata, R. and Reth, M. 1992. Crosslinking of the cell surface immunoglobulin (μ-surrogate light chains complex) on pre-B cells induces activation of V gene rearrangements at the immunoglobulin kappa locus. *Int. Immunol.* 4:637.